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Arthrobotrys nonseptata, a new anamorph from an *Orbilia* species

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Abstract — A new species of nematophagous fungi, *Arthrobotrys nonseptata,* was isolated from an unidentified *Orbilia* species. *Arthrobotrys nonseptata* produces simple, erect, unbranched conidiophores with conspicuous denticles at their tip, the conidia being nonseptate, ovoid to elongate ellipsoid. In the presence of nematodes, it forms three-dimensional adhesive networks.

Key words — nematode–trapping fungi, teleomorph–anamorph connection

Introduction

Nematophagous fungi destroy nematodes using several kinds of trapping devices: stalked and sessile adhesive knobs, two– or three– dimensional adhesive nets, and constricting and non-constricting hyphal rings. Those with adhesive nets are referred to form genus *Arthrobotrys* Corda which includes about 50 species (Scholler et al. 1999).

Pfister was the first to connect an *Arthrobotrys* species with a teleomorph of the ascomycetous genus *Orbilia* (1994). So far, five *Arthrobotrys* species have been linked with teleomorphs, including *O. auricolor* (A. Bloxam ex Berk.) Sacc. with four anamorphs, *A. oligospora* Fresen. and *A. cladodes* Drechsler (Pfister 1995), *A. yunnanensis* M.H. Mo & K.Q. Zhang (Mo et al. 2005), *A. psychrophila* (Drechsler) M. Scholler et al. (Rubner 1996, as *Monacrosporium pyschrophilum*); and *O. fimicola* Jeng & J.C. Krug and its anamorph *A. superba* Corda (Pfister 1994). In our survey of *Orbilia* species and their anamorphs, an *Arthrobotrys* species was isolated from an unidentified *Orbilia* species. This isolate could not be assigned to any previously described species and is described here as a new species, *Arthrobotrys nonseptata*.

* These authors contributed to this work equally.

Materials and methods

Collection of teleomorph, Isolation and characterization of the anamorph

Fresh specimens of an *Orbilia* species were collected on decaying bark of a broad-leaved tree, located in DaLongKou Park of Yimen County (N24°34', E101°00', at 1580 m altitude, in a coniferous-broadleaf forest including primarily *Cyclobalanopsis glaucoides* Schottky and *Pinus armandii* Franch.), Yunnan Province, China, on 18August, 2006 by Y. Zhang. A dried voucher specimen was deposited in the Laboratory for Conservation and Utilization of Bio–resource, Yunnan Province, China. (YMFT1.01852). To isolate its anamorph, several fresh apothecia were fixed to the lid of a Petri-dish with their hymenia upside down so that shooting ascospores were deposited on the surface of CMA (20 g corn meal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water). The Petri–dishes with apothecia were left 4–6 days at room temperature until deposits of ascospores were visible on the CMA. Agar blocks with deposits of ascospores were transferred to another CMA plate to avoid contamination. After incubation for 7–10 days at 25°C, conidiophores and conidia were observed. All cultures produced the same anamorph. Microscopic characters were observed and measured with an Olympus B51 microscope with differential interference contrast and a Zeiss Standard 20 microscope. Trapping organs were induced by adding about 100 nematodes (*Panagrellus redivivus* Goodey) to a 1 × 1 cm square slot at the margins of the colony created by removing the agar.

DNA extraction, PCR, and sequencing

Total DNA was isolated from fresh mycelium as described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the complete ITS (including 5.8S). The parameters for PCR amplifications were those used by Yu et al. (2006). The PCR products were purified with a commercial Kit (Bioteke Biotechnology Co., Ltd., China). Both strands were sequenced using the primers that were used for amplification. Sequencing was done on an LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase kit as described by Kindermann et al. (1998).

Phylogenetic analysis

We performed a parsimony analysis using ITS sequences of allied species of *Arthrobotrys*, and also of *Dactylellina* and *Drechslerella*. These latter species produce networks, adhesive knobs, and constricting rings, respectively. Genbank accession numbers are shown in our phylogenetic tree.

DNA sequences were aligned using ClustalX 1.83. Parsimony analysis was run in PAUP* 4.0b10 (Swofford 2002). Gaps were treated as missing data, all characters were equally weighted, initial 'MaxTrees' setting was 100, all trees were obtained by running the heuristic searches with tree-bisection-reconnection (TBR) as branch-swapping algorithm and up to 1000 random-addition sequence replications. To assess the relative support for each clade, bootstrap values were calculated from 10 replicate analyses with the heuristic search strategy and random addition sequence of the taxa.

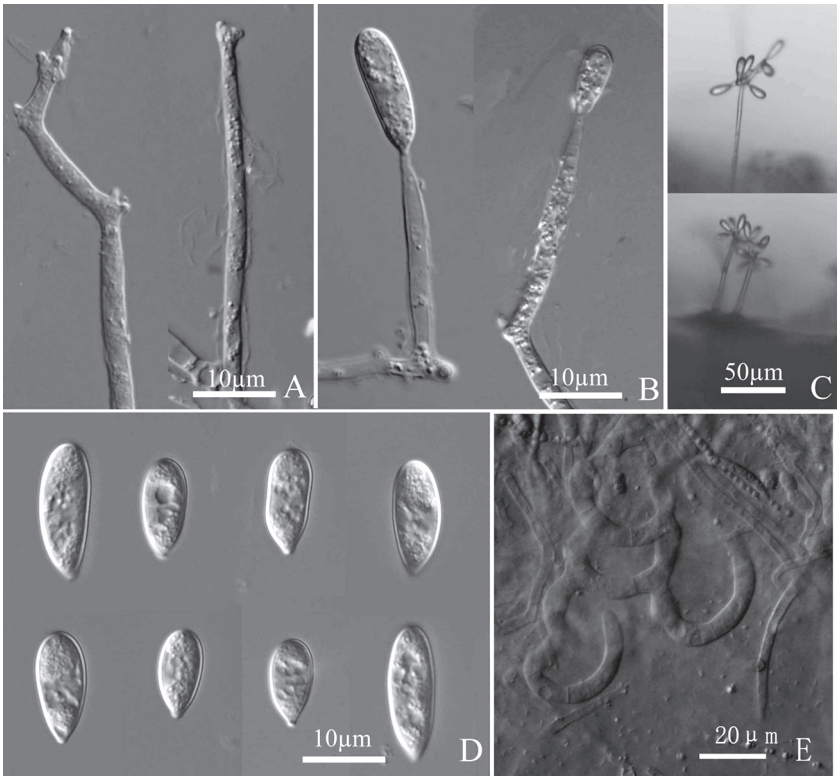

Plate 1. *Arthrobotrys nonseptata* (YMF1.01852) A. Conidiophores with short denticles. B. Conidium attached to a conidiophore. C. Conidiophores bearing conidia in clusters. D. Conidia. E. Adhesive nets.

Results

Taxonomic description

Arthrobotrys nonseptata Z.F. Yu, S.F. Li & K.Q. Zhang, **sp. nov.** Plate 1

MycoBank MB 512349

Coloniae in agaro albae, post 10 dies 25°C ad 35 mm diam. Mycelium sparsum, hyphis septatis, 3.5–4 μm latis. Conidiophora erecta, septata, non ramosa, 40–120 μm longa, 2–4 μm lata ad basim, 1.5–2 μm lata ad apicem, efferentia 3–10 conidia de retrogressis conidiogenis locis in latis perspicuis dendriculis in apice aut prope apicem. Conidia hyalina, elongate ellipsoidea, non-septata, 11–16.8 × 5–6.6 μm cum parvo truncatotubere in base. Reticula tenacia quae vermiculos nematodeos capiunt evolventivus.

Holotype: YMF 1.01852, permanent slide, Yimen County, DaLongkou Forest Park, Yunnan Province, PR China, Ze Fen Yu, Aug 18. 2006.

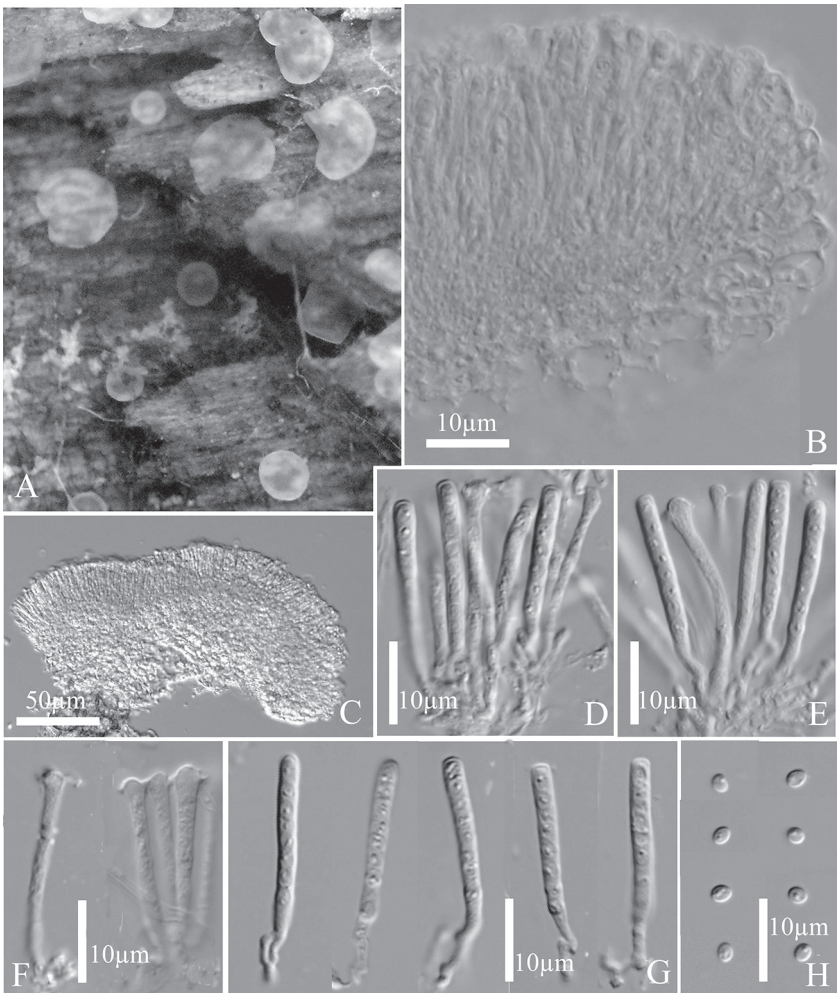

Plate 2. *Orbilia* sp. (YMFT 1.01852) A. Apothecia. B. Cells of ectal excipulum. C. Vertical section of part apothecia. D-E. Cluster of dead asci and paraphyses with living spores. F. Paraphyses. G. Asci. H. Ascospores.

Assumed teleomorph: an unidentified species of *Orbilia* (Plate 2), YMFT 1.01852, collected on decaying bark of a broad-leaved tree, DaLongKou Park, Yimen County, alt.1580 m, Yunnan Province, PR China, Ying Zhang. 18 August, 2006.

Etymology: The species epithet refers to the nonseptate conidia.

Colonies slow-growing on CMA medium, attaining less than 35 mm diam. in 10 days at 25°C. Vegetative hyphae hyaline, septate, 3.5–4 μm wide, aerial

mycelium sparse, hyaline, septate, branched, 2.5–4 µm wide. Conidiophores erect, septate, unbranched, 40–120 µm high, 2–4 µm wide in the lower part, 1.5–2 µm wide at the tip, producing 3–10 conidia from retrogressive conidiogenous loci on conspicuous denticles at and near the apex. Conidia hyaline, nonseptate, 11–16.8 × 5–6.6 µm, elongate ellipsoid, constricted at the base by forming a small truncate protuberance. Nematodes are captured by means of three-dimensional adhesive networks.

Phylogenetic analysis

Parsimony analysis of the ITS sequences yielded a single most parsimonious tree based on 192 parsimony-informative characters (147 constant characters, 206 uninformative characters). The MP tree had 890 steps in length with a consistency index (CI) of 0.6697 and a retention index (RI) of 0.5859. Our analysis used *Dactylella clavata* (a non-predacious member of the family *Orbiliaceae*) as outgroup. In the tree predacious species were divided into three clades. Strains with networks, constricting rings, and adhesive knobs formed A, B and C groups respectively, which is consistent with the results from Hagedorn & Scholler (1999) and Li et al. (2005). Our isolate of *A. nonseptata* falls into clade A, which is consistent with the production of adhesive networks.

Discussion

There are four known species of *Arthrobotrys* with nonseptate or occasionally uniseptate conidia. Conidia of *A. amerospora* (Schenck et al. 1977) are consistently nonseptate, while those of *A. anomala* (Barron & Davidson 1972), *A. botryospora* (Barron 1979) and *A. yunnanensis* (Mo et al. 2005) are occasionally uniseptate. *A. nonseptata* differs from these four species in conidial shape and size. *A. nonseptata* most closely resembles *A. yunnanensis* in regard to conidial shape. Both species were isolated from ascospores of *Orbilia* sp. The conidia of both are variable in shape and partly elongate or ellipsoid. In addition, the denticles of *A. yunnanensis* are longer than those of *A. nonseptata*. Other differences between *A. nonseptata* and the four known species are summarized in TABLE 1.

Results inferred from ITS sequence analyses support all five species discussed above in the same clade A. Although all have the same type of trapping device, the five species are distinct. Morphological and molecular characters serve to distinguish the species.

Arthrobotrys teleomorphs have, as far as known, narrow falcate ascospores usually referred to *O. auricolor* agg. However, the unidentified *Orbilia* species from which the present isolate derives has broadly ellipsoid ~2.5–3 µm long ascospores. In this character it resembles *O. orientalis* (Raitv.) Baral, which is

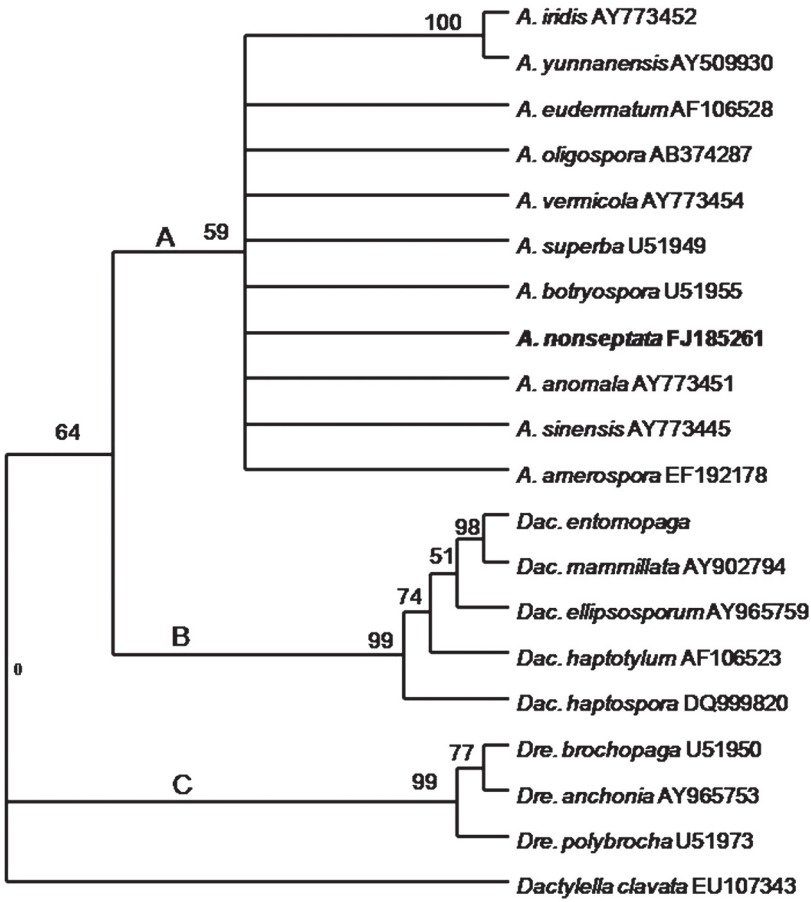

Plate 3. Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of some predacious species. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony-informative characters; only values >50% shown. Clade A includes members with adhesive nets; clade B includes members with constricting rings; clade C includes members with adhesive knobs.

connected to a *Drechslerella* anamorph with much longer, 3-septate conidia and constricting rings (Yu et al. 2006). For this reason, although *A. nonseptata* was isolated from the undescribed *Orbilia* collection, we hesitate to recognise them as an established anamorph–teleomorph pair. We hope that future isolations from this teleomorph will confirm or refute the relationship.

Table 1. Morphological comparison of *Arthrobotrys anomala*, *A. botryospora*, *A. amerospora*, *A. yunnanensis,* and *A. nonseptata*

Characters	*A. anomala*	*A. amerospora*	*A. botryospora*	*A. yunnanensis*	*A. nonseptata*
conidia					
Shape	cylindric to elongate ellipsoidal	obovoid	ellipsoidal	elongate, ellipsoid-cylindrical, or sl. clavate	elongate ellipsoidal
Size (μm)	13–22 × 3–7	15–31 × 10–20	12–20 × 11–15	17.5–32.5 × 2.75–7.5	11–16.8 × 5–6.6
Septation	0(–1)	0	0(–1)	0(–1)	0
conidiogenous cell					
Denticle	short	short	short	long	short
conidiophore					
Height (μm)	20–80	75–250	250–450	60–200	40–120

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